gm fresh weight) with 100 μl lysis buffer (containing 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, 1% SDS and 5 mM DTT) in a 1.5 ml Eppendorf tube. The mix was then ground with an electric drill as described above. Another 100 μl lysis buffer was added and the lysate was ground again briefly. The drill pestle was washed with 100 μl lysis buffer that was pooled with the lysate. After centrifugation at 14K at 4°C for 15 min. in a Beckman bench top centrifuge, the clear embryo lysate was mixed with 10 μl Dynal beads washed twice with lysis buffer. The suspension was incubated on ice for 5 min., with occasional mixing to allow binding of Poly (A) RNA to the oligo (dT) on the beads, and then left on a magnetic stand at room temperature for another 5 min. The liquid was removed and the beads were moved to a 0.2 ml PCR tube by suspending in 100 μl lysis buffer.

[0171] The beads were washed twice with 100 μl of washing buffer with LiDS and once with 50 μl of washing buffer. The mRNA was eluted from the beads in 6 μl water at 65°C for 2'. One μl T21VN primer (10 μM) and 1 μl SCSP oligo (cap switch primer, 5'-ctcttaattaagtacgcggg-3', 10 μM) were added to the mRNA eluate. The mixture was incubated at 70°C for 2' and cooled on ice. Three μl 5x First Strand Buffer, 1.5 μl DTT (20 mM), 1.5 μl dNTP (10 mM each) and 1 μl MMLV Superscript II (Gibco BRL) were added to the mRNA-primer mixture followed by incubation at 42°C for 1 h to synthesis first strand cDNAs. The cDNA was heated to 72°C for 1 min. to degrade RNA and then diluted to 100 μl with water. The lysis buffer, washing buffer and Dynal beads are components of the mRNA DIRECT kit (Dynal, NY). The first strand buffer (5x), 20

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

mM DTT and 10 mM dNTP are components of the SMART PCR cDNA synthesis kit (Clontech, Palo Alto, CA).

[0172] The first strand cDNAs synthesized as described above contains a T21VN sequence at their 5' ends and the SCSP sequence (see "SMARTTM cDNA, Clontech, Palo Alto, CA) at their 3' terminals. Total cDNA probes were made by PCR amplifying the first strand cDNAs using SMART cDNA PCR (Clontech, Palo Alto, CA) in the presence of labeling agent. Five 5 μl first strand cDNA solution was mixed with 5 μl 10x KlenTaq PCR buffer (Clonetech), 5 μl dATP + dGTP + dTTP (5 μM each), 1 μl T21VN primer, 1 μl SCSP oligo, 1 μl KlenTaq Mix, 5 μl 32P-dCTP (10 mCi/ml, Amersham) and 27 μl water. The PCR was performed using the setting of 94°C 2', 15 cycles of 95°C 15", 52°C 30", 68°C 6'. The PCR products were purified using NICK column (Pharmacia) according to the manufacture's instructions.

[0173] Currently, high-density array Southerns for both somatic and zygotic embryos at all the developmental stages have been performed. The dot array Southern data indicate that gene expression of late stage somatic embryos resembles middle stage zygotic embryos; many transcripts present during late zygotic embryogenesis (ZE) are absent in somatic embryos and late stage somatic embryo gene expression patterns resemble the patterns of middle stage zygotic embryos.

[0174] Cairney et al. (*In Vitro Cell. & Devel. Biol.- Plant*. 36:155-162 (2000); *Appl. Biochem. Biotech*. 77-79:5-17 (1999)) have discussed how this gene expression information may be used to improve the process of somatic embryogenesis; the references are incorporated in their entirety. As shown in Figure 2, the high-density array Southerns allows rapid evaluation of embryos subjected to protocol changes.

LAW OFFICES
FINNEGAN, HENDERSON, FARABOW, GARRETT, & DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

Following the expression of a known gene permits inferences about metabolism and is very valuable in developing media-improvement hypotheses. Further, detailed gene expression studies may help by providing an understanding of the timing and location of gene expression (e.g., *in situ* hybridization). The isolation of key genes also provides the ability to monitor the expression of these genes as stage specific markers and allows protocol variations to be quickly evaluated.

EXAMPLE 5: Identification of Markers for Superior Performance in Tissue Culture"

[0175] The evaluation of tissue culture modifications for pine somatic embryogenesis, depicted in Figure 8, is typically a lengthy process. However, where molecular tools are available, potentially improved media or genotypes can be discerned more rapidly, thereby avoiding the months of costly evaluation. (See Figure 8) Table 5 illustrates this proposition.

[0176] Table 4 describes several publicly available clones, *Lec*, *Fie*, and *Pkl*, used to provide a representative model for this example. Any clone within Table 1, SEQ ID NOS: 1-327, can be substituted for those in Table 4 to assay increased performance in tissue culture. Any promoter within Table 1, SEQ ID NOS: 328-334, can be incorporated with those in Table 4 or SEQ ID NOS: 1-327 to assay increased performance in tissue culture. In this scenario, Table 5, a representation of the information contained in Figure 9, shows performance of selected genotypes (260, 480, 499, and 500) in various media (1133 or 16) determined by the total number of embryos produced per medium as described by Pullman and Webb (1994), incorporated herein. Embryo maturation was determined by the presence of recognized morphology according to methods previously mentioned above. (Pullman and Webb, (1994))

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000